TRANSLATOR'S DECLARATION

I, Janet Hope, BSc(Hons.), MIL., MITI., translator to Messrs. Taylor and Meyer of 20 Kingsmead Road, London, SW2 3JD, Great Britain, verify that I know well both the German and the English language, that I have prepared the attached English translation of 39 pages of a German Patent application in the German language with the title:

Neue für das oxyR-Gen kodierende Nukleotidsequenzen

identified by the code number 000199 BT at the upper left of each page and that the attached English translation of this document is a true and correct translation of the document attached thereto to the best of my knowledge and belief.

I further declare that all statements made of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements are made with the knowledge that wilful false statements and the like are punishable by fine or imprisonment, or both, under 18 USC 1001, and that such false statements may jeopardize the validity of this document.

Signed: 

Dated: 10th December 2003

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The attached papers are a true and accurate reproduction of the original documents for this patent application.

Munich, 8th August 2001

On behalf of the President of the German Patent and Trade Mark Office

(signature)

Hiebinger

New nucleotide sequences which code for the oxyR gene

The invention provides nucleotide sequences from coryneform bacteria which code for the oxyR gene and a process for the fermentative preparation of amino acids, and a process for the fermentative preparation of amino acids, in particular L-lysine, using bacteria in which the oxyR gene is enhanced. The oxyR gene codes for the transcription regulator OxyR, which belongs to the LysR family.

Prior art

L-Amino acids, in particular L-lysine, are used in human medicine and in the pharmaceuticals industry, in the foodstuffs industry and very particularly in animal nutrition.

It is known that amino acids are prepared by fermentation from strains of coryneform bacteria, in particular Corynebacterium glutamicum. Because of their great importance, work is constantly being undertaken to improve the preparation processes. Improvements to the process can relate to fermentation measures, such as, for example, stirring and supply of oxygen, or the composition of the nutrient media, such as, for example, the sugar concentration during the fermentation, or the working up to the product form by, for example, ion exchange chromatography, or the intrinsic output properties of the microorganism itself.

Methods of mutagenesis, selection and mutant selection are used to improve the output properties of these microorganisms. Strains which are resistant to antimetabolites, such as e.g. the lysine analogue S-(2-aminoethyl)-cysteine, or are auxotrophic for metabolites of regulatory importance and produce L-lysine are obtained in this manner.

Methods of the recombinant DNA technique have also been employed for some years for improving the strain of Corynebacterium strains which produce L-amino acid, by amplifying individual amino acid biosynthesis genes and investigating the effect on the amino acid production.

Object of the invention

The inventors had the object of providing new measures for improved fermentative preparation of amino acids, in particular L-lysine.

Description of the invention

Where L-amino acids or amino acids are mentioned in the following, this means one or more amino acids, including their salts, chosen from the group consisting of L-asparagine, L-threonine, L-serine, L-glutamate, L-glycine, L-alanine, L-cysteine, L-valine, L-methionine, L-isoleucine, L-leucine, L-tyrosine, L-phenylalanine, L-histidine, L-lysine, L-tryptophan and L-arginine.

If L-lysine or lysine are mentioned in the following, this also means the salts, such as e.g. lysine monohydrochloride or lysine sulfate.

The invention provides an isolated polynucleotide from coryneform bacteria, comprising a polynucleotide sequence which codes for the oxyR gene chosen from the group consisting of

a) polynucleotide which is identical to the extent of at least 70 % to a polynucleotide which codes for a polypeptide which comprises the amino acid sequence of SEQ ID No. 2,

b) polynucleotide which codes for a polypeptide which comprises an amino acid sequence which is identical to

the extent of at least 70 % to the amino acid sequence of SEQ ID No.2,

c) polynucleotide which is complementary to the polynucleotides of a) or b), and

d) polynucleotide comprising at least 15 successive nucleotides of the polynucleotide sequence of a), b) or c),

the polypeptide preferably having the activity of the transcription regulator OxyR.

The invention also provides the abovementioned polynucleotide, this preferably being a DNA which is capable of replication, comprising:

(i) the nucleotide sequence, shown in SEQ ID No.1, or

(ii) at least one sequence which corresponds to sequence (i) within the range of the degeneration of the genetic code, or

(iii) at least one sequence which hybridizes with the sequence complementary to sequence (i) or (ii), and optionally

(iv) sense mutations of neutral function in (i).

The invention also provides

a polynucleotide comprising the nucleotide sequence as shown in SEQ ID No. 1;

a polynucleotide which codes for a polypeptide which comprises the amino acid sequence as shown in SEQ ID No. 2;

a vector containing the DNA sequence of C. glutamicum which codes for the oxyR gene, deposited in Corynebacterium glutamicum as pT-oxyRexp under DSM 13457, and

coryneform bacteria serving as the host cell, which contain the vector or in which the oxyR gene is enhanced.

The invention also provides polynucleotides which substantially comprise a polynucleotide sequence, which are obtainable by screening by means of hybridization of a corresponding gene library of a coryneform bacterium, which comprises the complete gene or parts thereof, with a probe which comprises the sequence of the polynucleotide according to the invention according to SEQ ID No.1 or a fragment thereof, and isolation of the polynucleotide sequence mentioned.

Polynucleotide sequences according to the invention are suitable as hybridization probes for RNA, cDNA and DNA, in order to isolate, in the full length, nucleic acids or polynucleotides or genes which code for the transcription regulator OxyR, or to isolate those nucleic acids or polynucleotides or genes which have a high similarity of sequence with that of the oxyR gene.

Polynucleotide sequences according to the invention are furthermore suitable as primers with the aid of which DNA of genes which code for the transcription regulator OxyR can be prepared with the polymerase chain reaction (PCR).

Such oligonucleotides which serve as probes or primers comprise at least 30, preferably at least 20, very particularly preferably at least 15 successive nucleotides. Oligonucleotides which have a length of at least 40 or 50 nucleotides are also suitable.

"Isolated" means separated out of its natural environment.

"Polynucleotide" in general relates to polyribonucleotides and polydeoxyribonucleotides, it being possible for these to be non-modified RNA or DNA or modified RNA or DNA.

The polynucleotides according to the invention include a polynucleotide according to SEQ ID No. 1 or a fragment prepared therefrom and also those which are at least 70 %, preferably at least 80 % and in particular at least 90 % to 95 % identical to the polynucleotide according to SEQ ID No. 1 or a fragment prepared therefrom.

"Polypeptides" are understood as meaning peptides or proteins which comprise two or more amino acids bonded via peptide bonds.

The polypeptides according to the invention include a polypeptide according to SEQ ID No. 2, in particular those with the biological activity of the transcription regulator OxyR, and also those which are at least 70 %, preferably at least 80 % and in particular which are at least 90 % to 95 % identical to the polypeptide according to SEQ ID No. 2 and have the activity mentioned.

The invention moreover provides a process for the fermentative preparation of amino acids, in particular L-lysine, using coryneform bacteria which in particular already produce amino acids, and in which the nucleotide sequences which code for the oxyR gene are enhanced, in particular over-expressed.

The term "enhancement" in this connection describes the increase in the intracellular activity of one or more enzymes in a microorganism which are coded by the corresponding DNA, for example by increasing the number of copies of the gene or genes, using a potent promoter or using a gene which codes for a corresponding enzyme having a high activity, and optionally combining these measures.

The microorganisms which the present invention provides can prepare L-amino acids, in particular L-lysine, from glucose, sucrose, lactose, fructose, maltose, molasses, starch, cellulose or from glycerol and ethanol. They can

be representatives of coryneform bacteria, in particular of the genus Corynebacterium. Of the genus Corynebacterium, there may be mentioned in particular the species Corynebacterium glutamicum, which is known among experts for its ability to produce L-amino acids.

Suitable strains of the genus Corynebacterium, in particular of the species Corynebacterium glutamicum (C.glutamicum), are in particular the known wild-type strains

Corynebacterium glutamicum ATCC13032
Corynebacterium acetoglutamicum ATCC15806
Corynebacterium acetoacidophilum ATCC13870
Corynebacterium thermoaminogenes FERM BP-1539
Corynebacterium melassecola ATCC17965
Brevibacterium flavum ATCC14067
Brevibacterium lactofermentum ATCC13869 and
Brevibacterium divaricatum ATCC14020

and L-lysine-producing mutants or strains prepared therefrom, such as, for example

Corynebacterium glutamicum FERM-P 1709
Brevibacterium flavum FERM-P 1708
Brevibacterium lactofermentum FERM-P 1712
Corynebacterium glutamicum FERM-P 6463
Corynebacterium glutamicum FERM-P 6464 and
Corynebacterium glutamicum DSM5715.

The inventors have succeeded in isolating the new oxyR gene of C. glutamicum which codes for the transcription regulator OxyR.

To isolate the oxyR gene or also other genes of C. glutamicum, a gene library of this microorganism is first set up in Escherichia coli (E. coli). The setting up of gene libraries is described in generally known textbooks and handbooks. The textbook by Winnacker: Gene und Klone,

Eine Einführung in die Gentechnologie (Verlag Chemie, Weinheim, Germany, 1990) or the handbook by Sambrook et al.: Molecular Cloning, A Laboratory Manual (Cold Spring Harbor Laboratory Press, 1989) may be mentioned as an example. A well-known gene library is that of the E. coli K-12 strain W3110 set up in λ vectors by Kohara et al. (Cell 50, 495-508 (1987)). Bathe et al. (Molecular and General Genetics, 252:255-265, 1996) describe a gene library of C. glutamicum ATCC13032, which was set up with the aid of the cosmid vector SuperCos I (Wahl et al., 1987, Proceedings of the National Academy of Sciences USA, 84:2160-2164) in the E. coli K-12 strain NM554 (Raleigh et al., 1988, Nucleic Acids Research 16:1563-1575).

Börmann et al. (Molecular Microbiology 6(3), 317-326) (1992)) in turn describe a gene library of C. glutamicum ATCC13032 using the cosmid pHC79 (Hohn and Collins, Gene 11, 291-298 (1980)). To prepare a gene library of C. glutamicum in E. coli it is also possible to use plasmids such as pBR322 (Bolivar, Life Sciences, 25, 807-818 (1979)) or pUC9 (Viera et al., 1982, Gene, 19:259-268). Suitable hosts are, in particular, those E. coli strains which are restriction- and recombination-defective. An example of these is the strain DH5αmcr, which has been described by Grant et al. (Proceedings of the National Academy of Sciences USA, 87 (1990) 4645-4649). The long DNA fragments cloned with the aid of cosmids can in turn be subcloned in the usual vectors suitable for sequencing and then sequenced, as is described e.g. by Sanger et al. (Proceedings of the National Academy of Sciences of the United States of America, 74:5463-5467, 1977).

The resulting DNA sequences can then be investigated with known algorithms or sequence analysis programs, such as e.g. that of Staden (Nucleic Acids Research 14, 217-232 (1986)),that of Marck (Nucleic Acids Research 16, 1829-1836

(1988)) or the GCG program of Butler (Methods of Biochemical Analysis 39, 74-97 (1998)).

The new DNA sequence of C. glutamicum which codes for the oxyR gene and which, as SEQ ID No. 1, is a constituent of the present invention has been found in this manner. The amino acid sequence of the corresponding protein has furthermore been derived from the present DNA sequence by the methods described above. The resulting amino acid sequence of the oxyR gene product is shown in SEQ ID No. 2.

Coding DNA sequences which result from SEQ ID No. 1 by the degeneracy of the genetic code are also a constituent of the invention. In the same way, DNA sequences which hybridize with SEQ ID No. 1 or parts of SEQ ID No. 1 are a constituent of the invention. Conservative amino acid exchanges, such as e.g. exchange of glycine for alanine or of aspartic acid for glutamic acid in proteins, are furthermore known among experts as "sense mutations" which do not lead to a fundamental change in the activity of the protein, i.e. are of neutral function. It is furthermore known that changes on the N and/or C terminus of a protein cannot substantially impair or can even stabilize the function thereof. Information in this context can be found by the expert, inter alia, in Ben-Bassat et al. (Journal of Bacteriology 169:751-757 (1987)), in O'Regan et al. (Gene 77:237-251 (1989)), in Sahin-Toth et al. (Protein Sciences 3:240-247 (1994)), in Hochuli et al. (Bio/Technology 6:1321-1325 (1988)) and in known textbooks of genetics and molecular biology. Amino acid sequences which result in a corresponding manner from SEQ ID No. 2 are also a constituent of the invention.

In the same way, DNA sequences which hybridize with SEQ ID No. 1 or parts of SEQ ID No. 1 are a constituent of the invention. Finally, DNA sequences which are prepared by the polymerase chain reaction (PCR) using primers which result from SEQ ID No. 1 are a constituent of the

invention. Such oligonucleotides typically have a length of at least 15 nucleotides.

Instructions for identifying DNA sequences by means of hybridization can be found by the expert, inter alia, in the handbook "The DIG System Users Guide for Filter Hybridization" from Boehringer Mannheim GmbH (Mannheim, Germany, 1993) and in Liebl et al. (International Journal of Systematic Bacteriology (1991) 41: 255-260). The hybridization takes place under stringent conditions, that is to say only hybrids in which the probe and target sequence, i.e. the polynucleotides treated with the probe, are at least 70 % identical are formed. It is known that the stringency of the hybridization, including the washing steps, is influenced or determined by varying the buffer composition, the temperature and the salt concentration. The hybridization reaction is preferably carried out under a relatively low stringency compared with the washing steps (Hybaid Hybridisation Guide, Hybaid Limited, Teddington, UK, 1996).

A 5x SSC buffer at a temperature of approx. 50 - 68°C, for example, can be employed for the hybridization reaction. Probes can also hybridize here with polynucleotides which are less than 70 % identical to the sequence of the probe. Such hybrids are less stable and are removed by washing under stringent conditions. This can be achieved, for example, by lowering the salt concentration to 2x SSC and subsequently 0.5x SSC (The DIG System User's Guide for Filter Hybridisation, Boehringer Mannheim, Mannheim, Germany, 1995), a temperature of approx. 50 - 68°C being established. It is optionally possible to lower the salt concentration to 0.1x SSC. Polynucleotide fragments which are, for example, at least 70 % or at least 80 % or at least 90 % to 95 % identical to the sequence of the probe employed can be isolated by increasing the hybridization temperature stepwise in steps of approx. 1 - 2°C. Further

instructions on hybridization are obtainable on the market in the form of so-called kits (e.g. DIG Easy Hyb from Roche Diagnostics GmbH, Mannheim, Germany, Catalogue No. 1603558).

Instructions for amplification of DNA sequences with the aid of the polymerase chain reaction (PCR) can be found by the expert, inter alia, in the handbook by Gait: Oligonucleotide synthesis: A Practical Approach (IRL Press, Oxford, UK, 1984) and in Newton and Graham: PCR (Spektrum Akademischer Verlag, Heidelberg, Germany, 1994).

It has been found that coryneform bacteria produce amino acids, in particular L-lysine, in an improved manner after over-expression of the oxyR gene.

To achieve an over-expression, the number of copies of the corresponding genes can be increased, or the promoter and regulation region or the ribosome binding site upstream of the structural gene can be mutated. Expression cassettes which are incorporated upstream of the structural gene act in the same way. By inducible promoters, it is additionally possible to increase the expression in the course of fermentative L-lysine production. The expression is likewise improved by measures to prolong the life of the m-RNA. Furthermore, the enzyme activity is also increased by preventing the degradation of the enzyme protein. The genes or gene constructs can either be present in plasmids with a varying number of copies, or can be integrated and amplified in the chromosome. Alternatively, an over-expression of the genes in question can furthermore be achieved by changing the composition of the media and the culture procedure.

Instructions in this context can be found by the expert, inter alia, in Martin et al. (Bio/Technology 5, 137-146 (1987)), in Guerrero et al. (Gene 138, 35-41 (1994)), Tsuchiya and Morinaga (Bio/Technology 6, 428-430 (1988)),

in Eikmanns et al. (Gene 102, 93-98 (1991)), in European Patent Specification 0 472 869, in US Patent 4,601,893, in Schwarzer and Pühler (Bio/Technology 9, 84-87 (1991), in Reinscheid et al. (Applied and Environmental Microbiology 60, 126-132 (1994)), in LaBarre et al. (Journal of Bacteriology 175, 1001-1007 (1993)), in Patent Application WO 96/15246, in Malumbres et al. (Gene 134, 15 - 24 (1993)), in Japanese Laid-Open Specification JP-A-10-229891, in Jensen and Hammer (Biotechnology and Bioengineering 58, 191-195 (1998)), in Makrides (Microbiological Reviews 60:512-538 (1996)) and in known textbooks of genetics and molecular biology.

By way of example, for enhancement the oxyR gene according to the invention was over-expressed with the aid of episomal plasmids. Suitable plasmids are those which are replicated in coryneform bacteria. Numerous known plasmid vectors, such as e.g. pZ1 (Menkel et al., Applied and Environmental Microbiology (1989) 64: 549-554), pEKEx1 (Eikmanns et al., Gene 102:93-98 (1991)) or pHS2-1 (Sonnen et al., Gene 107:69-74 (1991)) are based on the cryptic plasmids pHM1519, pBL1 or pGA1. Other plasmid vectors, such as e.g. those based on pCG4 (US-A 4,489,160) or pNG2 (Serwold-Davis et al., FEMS Microbiology Letters 66, 119-124 (1990)) or pAG1 (US-A 5,158,891), can be used in the same manner.

An example of a plasmid with the aid of which the oxyR gene can be over-expressed is the E.coli-C.glutamicum shuttle vector pT-oxyRexp. It contains the replication region rep of the plasmid pGA1 including the replication effector per (US-A- 5,175,108; Nesvera et al., Journal of Bacteriology 179, 1525-1532 (1997)), the tetracycline resistance-imparting tetA(Z) gene of the plasmid pAG1 (US-A-5,158,891; gene library entry at the National Center for Biotechnology Information (NCBI, Bethesda, MD, USA) with the accession number AF121000), the replication origin oriV

of the plasmid pMB1 (Sutcliffe, Cold Spring Harbor Symposium on Quantitative Biology 43, 77-90 (1979)), the lacZα gene fragment including the lac promoter and a multiple cloning site (mcs) (Norrander, J.M. et al. Gene 26, 101-106 (1983)) and the mob region of the plasmid RP4 (Simon et al.,(1983) Bio/Technology 1:784-791).

The plasmid pT-oxyRexp is shown in figure 2.

Plasmid vectors which are furthermore suitable are also those with the aid of which the process of gene amplification by integration into the chromosome can be used, as has been described, for example, by Reinscheid et al. (Applied and Environmental Microbiology 60, 126-132 (1994)) for duplication or amplification of the hom-thrB operon. In this method, the complete gene is cloned in a plasmid vector which can replicate in a host (typically E. coli), but not in C. glutamicum. Possible vectors are, for example, pSUP301 (Simon et al., Bio/Technology 1, 784-791 (1983)), pK18mob or pK19mob (Schäfer et al., Gene 145, 69-73 (1994)), pGEM-T (Promega corporation, Madison, WI, USA), pCR2.1-TOPO (Shuman (1994). Journal of Biological Chemistry 269:32678-84; US-A 5,487,993), pCR®Blunt (Invitrogen, Groningen, Holland; Bernard et al., Journal of Molecular Biology, 234: 534-541 (1993)), pEM1 (Schrumpf et al, 1991, Journal of Bacteriology 173:4510-4516) or pBGS8 (Spratt et al.,1986, Gene 41: 337-342). The plasmid vector which contains the gene to be amplified is then transferred into the desired strain of C. glutamicum by conjugation or transformation. The method of conjugation is described, for example, by Schäfer et al. (Applied and Environmental Microbiology 60, 756-759 (1994)). Methods for transformation are described, for example, by Thierbach et al. (Applied Microbiology and Biotechnology 29, 356-362 (1988)), Dunican and Shivnan (Bio/Technology 7, 1067-1070 (1989)) and Tauch et al. (FEMS Microbiological Letters 123, 343-347 (1994)). After homologous recombination by means

of a "cross over" event, the resulting strain contains at least two copies of the gene in question.

In addition, it may be advantageous for the production of amino acids, in particular L-lysine, to enhance one or more enzymes of the particular biosynthesis pathway, of glycolysis, of anaplerosis, of the pentose phosphate cycle, of the citric acid cycle or of amino acid export and optionally regulatory proteins, in addition to the oxyR gene.

Thus, for example, for the preparation of amino acids, in particular L-lysine, one or more genes chosen from the group consisting of

- the dapA gene which codes for dihydrodipicolinate synthase (EP-B 0 197 335),
- the gap gene which codes for glyceraldehyde 3-phosphate dehydrogenase (Eikmanns (1992). Journal of Bacteriology 174:6076-6086),
- the tpi gene which codes for triose phosphate isomerase (Eikmanns (1992). Journal of Bacteriology 174:6076-6086),
- the pgk gene which codes for 3-phosphoglycerate kinase (Eikmanns (1992). Journal of Bacteriology 174:6076-6086),
- the pyc gene which codes for pyruvate carboxylase (Peters-Wendisch et al.(Microbiology 144, 915 - 927 (1998)),
- the lysC gene which codes for a feed back resistant aspartate kinase (Kalinowski et al. (1990), Molecular and General Genetics 224, 317-324; Accession No.P26512),
- the lysE gene which codes for lysine export (DE-A-195 48 222)

- the mqo gene which codes for malate-quinone oxidoreductase (Molenaar et al. (1998), European Journal of Biochemistry 254: 395-403),
- the zwf gene which codes for glucose 6-phosphate dehydrogenase (JP-A-09224661),
- the gnd gene which codes for 6-phosphogluconate dehydrogenase (US: 09/531,265),
- the sod gene which codes for superoxide dismutase (US: 09/373,731),
- the zwa1 gene which codes for the Zwa1 protein (DE: 199 59 328.0, DSM 13115)

can be enhanced, in particular over-expressed.

It may furthermore be advantageous for the production of amino acids, in particular L-lysine, in addition to the enhancement of the oxyR gene, for one or more genes chosen from the group consisting of

- the pck gene which codes for phosphoenol pyruvate carboxykinase (DE: 199 50 409.1, DSM 13047),
- the pgi gene which codes for glucose 6-phosphate isomerase (US: 09/396,478, DSM 12969),
- the poxB gene which codes for pyruvate oxidase (DE: 199 51 975.7, DSM 13114),
- the zwa2 gene which codes for the Zwa2 protein (DE: 199 59 327.2, DSM 13113)

to be attenuated, in particular for the expression thereof to be reduced.

In addition to over-expression of the oxyR gene it may furthermore be advantageous, for the production of amino acids, in particular L-lysine, to eliminate undesirable

side reactions, (Nakayama: "Breeding of Amino Acid Producing Micro-organisms", in: Overproduction of Microbial Products, Krumphanzl, Sikyta, Vanek (eds.), Academic Press, London, UK, 1982).

The microorganisms prepared according to the invention can be cultured continuously or discontinuously in the batch process (batch culture) or in the fed batch (feed process) or repeated fed batch process (repetitive feed process) for the purpose of production of amino acids, in particular L-lysine. A summary of known culture methods are described in the textbook by Chmiel (Bioprozesstechnik 1. Einführung in die Bioverfahrenstechnik (Gustav Fischer Verlag, Stuttgart, 1991)) or in the textbook by Storhas (Bioreaktoren und periphere Einrichtungen (Vieweg Verlag, Braunschweig/Wiesbaden, 1994)).

The culture medium to be used must meet the requirements of the particular strains in a suitable manner. Descriptions of culture media for various microorganisms are contained in the handbook "Manual of Methods for General Bacteriology" of the American Society for Bacteriology (Washington D.C., USA, 1981).

Sugars and carbohydrates, such as e.g. glucose, sucrose, lactose, fructose, maltose, molasses, starch and cellulose, oils and fats, such as e.g. soya oil, sunflower oil, groundnut oil and coconut fat, fatty acids, such as e.g. palmitic acid, stearic acid and linoleic acid, alcohols, such as e.g. glycerol and ethanol, and organic acids, such as e.g. acetic acid, can be used as the source of carbon. These substance can be used individually or as a mixture.

Organic nitrogen-containing compounds, such as peptones, yeast extract, meat extract, malt extract, corn steep liquor, soya bean flour and urea, or inorganic compounds, such as ammonium sulfate, ammonium chloride, ammonium phosphate, ammonium carbonate and ammonium nitrate, can be

used as the source of nitrogen. The sources of nitrogen can be used individually or as a mixture.

Phosphoric acid, potassium dihydrogen phosphate or dipotassium hydrogen phosphate or the corresponding sodium-containing salts can be used as the source of phosphorus. The culture medium must furthermore comprise salts of metals, such as e.g. magnesium sulfate or iron sulfate, which are necessary for growth. Finally, essential growth substances, such as amino acids and vitamins, can be employed in addition to the abovementioned substances. Suitable precursors can moreover be added to the culture medium. The starting substances mentioned can be added to the culture in the form of a single batch, or can be fed in during the culture in a suitable manner.

Basic compounds, such as sodium hydroxide, potassium hydroxide, ammonia or aqueous ammonia, or acid compounds, such as phosphoric acid or sulfuric acid, can be employed in a suitable manner to control the pH of the culture. Antifoams, such as e.g. fatty acid polyglycol esters, can be employed to control the development of foam. Suitable substances having a selective action, such as e.g. antibiotics, can be added to the medium to maintain the stability of plasmids. To maintain aerobic conditions, oxygen or oxygen-containing gas mixtures, such as e.g. air, are introduced into the culture. The temperature of the culture is usually 20°C to 45°C, and preferably 25°C to 40°C. Culturing is continued until a maximum of lysine has formed. This target is usually reached within 10 hours to 160 hours.

The analysis of L-lysine can be carried out by ion exchange chromatography with subsequent ninhydrin derivatization, as described by Spackman et al. (Analytical Chemistry, 30, (1958), 1190).

The following microorganism has been deposited at the Deutsche Sammlung für Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany) in accordance with the Budapest Treaty:

- Corynebacterium glutamicum strain DSM5715/pT-oxyRexp as DSM 13457.

The process according to the invention is used for the fermentative preparation of amino acids, in particular L-lysine.

The present invention is explained in more detail in the following with the aid of embodiment examples.

The isolation of plasmid DNA from Escherichia coli and all techniques of restriction, Klenow and alkaline phosphatase treatment were carried out by the method of Sambrook et al. (Molecular Cloning. A Laboratory Manual (1989) Cold Spring Harbour Laboratory Press, Cold Spring Harbor, NY, USA). Methods for transformation of Escherichia coli are also described in this handbook.

The composition of the usual nutrient media, such as LB or TY medium, can also be found in the handbook by Sambrook et al.

Example 1

Preparation of a genomic cosmid gene library from Corynebacterium glutamicum ATCC 13032

Chromosomal DNA from Corynebacterium glutamicum ATCC 13032 was isolated as described by Tauch et al. (1995, Plasmid 33:168-179) and partly cleaved with the restriction enzyme Sau3AI (Amersham Pharmacia, Freiburg, Germany, Product Description Sau3AI, Code no. 27-0913-02). The DNA fragments were dephosphorylated with shrimp alkaline phosphatase (Roche Diagnostics GmbH, Mannheim, Germany,

Product Description SAP, Code no. 1758250). The DNA of the cosmid vector SuperCos1 (Wahl et al. (1987) Proceedings of the National Academy of Sciences USA 84:2160-2164), obtained from Stratagene (La Jolla, USA, Product Description SuperCos1 Cosmid Vector Kit, Code no. 251301) was cleaved with the restriction enzyme XbaI (Amersham Pharmacia, Freiburg, Germany, Product Description XbaI, Code no. 27-0948-02) and likewise dephosphorylated with shrimp alkaline phosphatase.

The cosmid DNA was then cleaved with the restriction enzyme BamHI (Amersham Pharmacia, Freiburg, Germany, Product Description BamHI, Code no. 27-0868-04). The cosmid DNA treated in this manner was mixed with the treated ATCC13032 DNA and the batch was treated with T4 DNA ligase (Amersham Pharmacia, Freiburg, Germany, Product Description T4-DNA-Ligase, Code no.27-0870-04). The ligation mixture was then packed in phages with the aid of Gigapack II XL Packing Extract (Stratagene, La Jolla, USA, Product Description Gigapack II XL Packing Extract, Code no. 200217).

For infection of the E. coli strain NM554 (Raleigh et al. 1988, Nucleic Acid Research 16:1563-1575) the cells were taken up in 10 mM $MgSO_4$ and mixed with an aliquot of the phage suspension. The infection and titering of the cosmid library were carried out as described by Sambrook et al. (1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor), the cells being plated out on LB agar (Lennox, 1955, Virology, 1:190) with 100 mg/l ampicillin. After incubation overnight at 37°C, recombinant individual clones were selected.

Example 2

Isolation and sequencing of the oxyR gene

The cosmid DNA of an individual colony was isolated with the Qiaprep Spin Miniprep Kit (Product No. 27106, Qiagen,

Hilden, Germany) in accordance with the manufacturer's instructions and partly cleaved with the restriction enzyme Sau3AI (Amersham Pharmacia, Freiburg, Germany, Product Description Sau3AI, Product No. 27-0913-02). The DNA fragments were dephosphorylated with shrimp alkaline phosphatase (Roche Diagnostics GmbH, Mannheim, Germany, Product Description SAP, Product No. 1758250). After separation by gel electrophoresis, the cosmid fragments in the size range of 1500 to 2000 bp were isolated with the QiaExII Gel Extraction Kit (Product No. 20021, Qiagen, Hilden, Germany).

The DNA of the sequencing vector pZero-1, obtained from Invitrogen (Groningen, Holland, Product Description Zero Background Cloning Kit, Product No. K2500-01), was cleaved with the restriction enzyme BamHI (Amersham Pharmacia, Freiburg, Germany, Product Description BamHI, Product No. 27-0868-04). The ligation of the cosmid fragments in the sequencing vector pZero-1 was carried out as described by Sambrook et al. (1989, Molecular Cloning: A laboratory Manual, Cold Spring Harbor), the DNA mixture being incubated overnight with T4 ligase (Pharmacia Biotech, Freiburg, Germany). This ligation mixture was then electroporated (Tauch et al. 1994, FEMS Microbiol Letters, 123:343-7) into the E. coli strain DH5αMCR (Grant, 1990, Proceedings of the National Academy of Sciences U.S.A., 87:4645-4649) and plated out on LB agar (Lennox, 1955, Virology, 1:190) with 50 mg/l zeocin.

The plasmid preparation of the recombinant clones was carried out with the Biorobot 9600 (Product No. 900200, Qiagen, Hilden, Germany). The sequencing was carried out by the dideoxy chain termination method of Sanger et al. (1977, Proceedings of the National Academy of Sciences U.S.A., 74:5463-5467) with modifications according to Zimmermann et al. (1990, Nucleic Acids Research, 18:1067). The "RR dRhodamin Terminator Cycle Sequencing Kit" from PE

Applied Biosystems (Product No. 403044, Weiterstadt, Germany) was used. The separation by gel electrophoresis and analysis of the sequencing reaction were carried out in a "Rotiphoresis NF Acrylamide/Bisacrylamide" Gel (29:1) (Product No. A124.1, Roth, Karlsruhe, Germany) with the "ABI Prism 377" sequencer from PE Applied Biosystems (Weiterstadt, Germany).

The raw sequence data obtained were then processed using the Staden program package (1986, Nucleic Acids Research, 14:217-231) version 97-0. The individual sequences of the pZero1 derivatives were assembled to a continuous contig. The computer-assisted coding region analysis was prepared with the XNIP program (Staden, 1986, Nucleic Acids Research, 14:217-231).

The resulting nucleotide sequence is shown in SEQ ID No. 1. Analysis of the nucleotide sequence showed an open reading frame of 981 base pairs, which was called the oxyR gene. The oxyR gene codes for a protein of 327 amino acids.

Example 3

Preparation of a shuttle vector pT-oxyRexp for enhancement of the oxyR gene in C. glutamicum

3.1. Cloning of the oxyR gene

From the strain ATCC 13032, chromosomal DNA was isolated by the method of Eikmanns et al. (Microbiology 140: 1817 -1828 (1994)). On the basis of the sequence of the oxyR gene known for C. glutamicum from example 2, the following oligonucleotides were chosen for the polymerase chain reaction:

OxyR (oxy-exp):

5` GAT CGA GAA TTC AAA GGA AGA TCA GCT TAG 3`

OxyR (oxy R2):

5` GGA AAA CCT CTA GAA AAA CT 3`

The primers shown were synthesized by ARK Scientific GmbH Biosystems (Darmstadt, Germany) and the PCR reaction was carried out by the standard PCR method of Innis et al. (PCR protocols. A guide to methods and applications, 1990, Academic Press) with Pwo-Polymerase from Roche Diagnostics GmbH (Mannheim, Germany). With the aid of the polymerase chain reaction, the primers allow amplification of a DNA fragment approx. 1.43 kb in size, which carries the oxyR gene. Furthermore, the primer OxyR (oxy-exp) contains the sequence for the cleavage site of the restriction endonuclease EcoRI, and the primer OxyR (oxy R2) the cleavage site of the restriction endonuclease XbaI, which are marked by underlining in the nucleotide sequence shown above.

The amplified DNA fragment of approx. 1.43 kb which carries the oxyR gene was ligated with the Zero Blunt™ Kit of Invitrogen Corporation (Carlsbad, CA, USA; Catalogue Number K2700-20) in the vector pCR®Blunt II (Bernard et al., Journal of Molecular Biology, 234: 534-541 (1993)). The E. coli strain Top10 (Grant et al., Proceedings of the National Academy of Sciences USA, 87 (1990) 4645-4649) was then transformed with the ligation batch in accordance with the instructions of the manufacturer of the kit (Invitrogen Corporation,Carlsbad, CA, USA). Selection of plasmid-carrying cells was carried out by plating out the transformation batch on LB Agar (Sambrook et al., Molecular cloning: a laboratory manual. 2nd Ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989), which had been supplemented with 25 mg/l kanamycin. Plasmid DNA was isolated from a transformant with the aid of the QIAprep Spin Miniprep Kit from Qiagen (Hilden, Germany) and checked by treatment with the restriction enzyme XbaI and EcoRI with subsequent agarose gel electrophoresis (0.8 %). The DNA sequence of the amplified DNA fragment was checked by sequencing. The plasmid was called pCR-oxyRexp. The strain was called E. coli Top10 / pCR-oxyRexp.

3.2. Preparation of the E. coli - C. glutamicum shuttle vector pEC-T18mob2

The E. coli - C. glutamicum shuttle vector was constructed according to the prior art. The vector contains the replication region rep of the plasmid pGA1 including the replication effector per (US-A- 5,175,108; Nesvera et al., Journal of Bacteriology 179, 1525-1532 (1997)), the tetracycline resistance-imparting tetA(Z) gene of the plasmid pAG1 (US-A- 5,158,891; gene library entry at the National Center for Biotechnology Information (NCBI, Bethesda, MD, USA) with the accession number AF121000), the replication region oriV of the plasmid pMB1 (Sutcliffe, Cold Spring Harbor Symposium on Quantitative Biology 43, 77-90 (1979)), the lacZα gene fragment including the lac promoter and a multiple cloning site (mcs) (Norrander, J.M. et al. Gene 26, 101-106 (1983)) and the mob region of the plasmid RP4 (Simon et al.,(1983) Bio/Technology 1:784-791). The vector constructed was transformed in the E. coli strain DH5α (Hanahan, In: DNA cloning. A Practical Approach. Vol. I. IRL-Press, Oxford, Washington DC, USA). Selection of plasmid-carrying cells was carried out by plating out the transformation batch on LB Agar (Sambrook et al., Molecular cloning: a laboratory manual. 2nd Ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.), which had been supplemented with 5 mg/l tetracycline. Plasmid DNA was isolated from a transformant with the aid of the QIAprep Spin Miniprep Kit from Qiagen and checked by restriction with the restriction enzyme EcoRI and HindIII subsequent agarose gel electrophoresis (0.8 %). The plasmid was called pEC-T18mob2 and is shown in figure 1.

The following microorganism has been deposited at the Deutsche Sammlung für Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany) in accordance with the Budapest Treaty:

- Escherichia coli strain DH5α/pEC-T18mob2 as DSM 13244

3.3. Cloning of oxyR in the E. coli-C. glutamicum shuttle vector pEC-T18mob2

The E. coli - C. glutamicum shuttle vector pEC-T18mob2 described in example 3.2 was used as the vector. DNA of this plasmid was cleaved completely with the restriction enzymes EcoRI and XbaI and then dephosphorylated with shrimp alkaline phosphatase (Roche Diagnostics GmbH, Mannheim, Germany, Product Description SAP, Product No. 1758250).

The oxyR gene was isolated from the plasmid pCR-oxyRexp described in example 3.1. by complete cleavage with the enzymes EcoRI and XbaI. The oxyR fragment approx. 1400bp in size was isolated from the agarose gel with the QiaExII Gel Extraction Kit (Product No. 20021, Qiagen, Hilden, Germany).

The oxyR fragment obtained in this manner was mixed with the prepared vector pEC-T18mob2 and the batch was treated with T4 DNA ligase (Amersham Pharmacia, Freiburg, Germany, Product Description T4-DNA-Ligase, Code no. 27-0870-04). The ligation batch was transformed in the E. coli strain DH5α (Hanahan, In: DNA cloning. A Practical Approach. Vol. I. IRL-Press, Oxford, Washington DC, USA). Selection of plasmid-carrying cells was made by plating out the transformation batch on LB agar (Lennox, 1955, Virology, 1:190) with 5 mg/l tetracycline. After incubation overnight at 37°C, recombinant individual clones were selected. Plasmid DNA was isolated from a transformant with the Qiaprep Spin Miniprep Kit (Product No. 27106, Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions and cleaved with the restriction enzymes EcoRI and XbaI to check the plasmid by subsequent agarose gel electrophoresis. The resulting plasmid was called pT-oxyRexp. It is shown in figure 2.

Example 4

Transformation of the strain DSM5715 with the plasmid pT-oxyRexp

The strain DSM5715 was transformed with the plasmid pT-oxyRexp using the electroporation method described by Liebl et al., (FEMS Microbiology Letters, 53:299-303 (1989)). Selection of the transformants took place on LBHIS agar comprising 18.5 g/l brain-heart infusion broth, 0.5 M sorbitol, 5 g/l Bacto-tryptone, 2.5 g/l Bacto-yeast extract, 5 g/l NaCl and 18 g/l Bacto-agar, which had been supplemented with 5 mg/l tetracycline. Incubation was carried out for 2 days at 33°C.

Plasmid DNA was isolated from a transformant by conventional methods (Peters-Wendisch et al., 1998, Microbiology, 144, 915 -927), cleaved with the restriction endonucleases EcoRI and XbaI, and the plasmid was checked by subsequent agarose gel electrophoresis. The resulting strain was called DSM5715/pT-oxyRexp.

Example 5

Preparation of lysine

The C. glutamicum strain DSM5715/pT-oxyRexp obtained in example 4 was cultured in a nutrient medium suitable for the production of lysine and the lysine content in the culture supernatant was determined.

For this, the strain was first incubated on an agar plate with the corresponding antibiotic (brain-heart agar with tetracycline (5 mg/l)) for 24 hours at 33°C. Starting from this agar plate culture, a preculture was seeded (10 ml medium in a 100 ml conical flask). The complete medium CgIII was used as the medium for the preculture.

Medium Cg III

NaCl	2.5 g/l
Bacto-Peptone	10 g/l
Bacto-Yeast extract	10 g/l
Glucose (autoclaved separately)	2 % (w/v)

The pH was brought to pH 7.4

Tetracycline (5 mg/l) was added to this. The preculture was incubated for 16 hours at 33°C at 240 rpm on a shaking machine. A main culture was seeded from this preculture such that the initial OD (660 nm) of the main culture was 0.05. Medium MM was used for the main culture.

Medium MM

CSL (corn steep liquor)	5 g/l
MOPS (morpholinopropanesulfonic acid)	20 g/l
Glucose (autoclaved separately)	50 g/l
$(NH_4)_2SO_4$	25 g/l
KH_2PO_4	0.1 g/l
$MgSO_4 * 7 H_2O$	1,0 g/l
$CaCl_2 * 2 H_2O$	10 mg/l
$FeSO_4 * 7 H_2O$	10 mg/l
$MnSO_4 * H_2O$	5.0mg/l
Biotin (sterile-filtered)	0.3 mg/l

Thiamine * HCl (sterile-filtered)	0.2 mg/l
L-Leucine (sterile-filtered)	0.1 g/l
$CaCO_3$	25 g/l

The CSL, MOPS and the salt solution were brought to pH 7 with aqueous ammonia and autoclaved. The sterile substrate and vitamin solutions were then added, as well as the $CaCO_3$ autoclaved in the dry state.

Culturing is carried out in a 10 ml volume in a 100 ml conical flask with baffles. Tetracycline (5 mg/l) was added. Culturing was carried out at 33°C and 80 % atmospheric humidity.

After 72 hours, the OD was determined at a measurement wavelength of 660 nm with a Biomek 1000 (Beckmann Instruments GmbH, Munich). The amount of lysine formed was determined with an amino acid analyzer from Eppendorf-BioTronik (Hamburg, Germany) by ion exchange chromatography and post-column derivatization with ninhydrin detection.

The result of the experiment is shown in table 1.

Table 1

Strain	OD(660)	Lysine HCl g/l
DSM5715	6.8	13.68
DSM5715/pT-oxyRexp	6.5	14.73

The following figures are attached:

Figure 1: Map of the plasmid pEC-T18mob2

Figure 2: Map of the plasmid pT-oxyRexp

The abbreviations and designations used have the following meaning:

per:	Gene for control of the number of copies from pGA1
oriV:	ColE1-similar origin from pMB1
rep:	Plasmid-coded replication region from C. glutamicum plasmid pGA1
RP4mob:	RP4 mobilization site
lacZ-alpha:	lacZ gene fragment from E.coli
Tet:	Resistance gene for tetracycline
oxyR:	oxyR gene of C.glutamicum
EcoRI:	Cleavage site of the restriction enzyme EcoRI
Ecl136II:	Cleavage site of the restriction enzyme Ecl136II
HindIII:	Cleavage site of the restriction enzyme HindIII
KpnI:	Cleavage site of the restriction enzyme KpnI
SalI:	Cleavage site of the restriction enzyme SalI
SmaI:	Cleavage site of the restriction enzyme SmaI
PstI:	Cleavage site of the restriction enzyme PstI
BamHI:	Cleavage site of the restriction enzyme BamHI
XbaI:	Cleavage site of the restriction enzyme XbaI
XmaI:	Cleavage site of the restriction enzyme XmaI
XhoI:	Cleavage site of the restriction enzyme XhoI
PstI:	Cleavage site of the restriction enzyme PstI

SEQUENCE PROTOCOL

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caggtggtga aggatccaga aggtgataat tccttcgcgt ttgttgccac cattgatctt 300

gatgcctctg atgatgcagg tgaggtgcgt tttggatcgc tgtcgattga ccacaactag 360

gggtttgcgt cgaaaagcaa gcacgcctgg tgcctgattt gagcggtttt acctatggcg 420

ctttggcgcc gtcaaactgt cccagcgatt tcattattat tttcgtgcat tcaccgttat 480

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Arg Thr Phe Val Thr Ile Ala Glu Cys Lys His Phe Gly Thr Ala Ala
15 20 25

acc aag ctg tcc att tcg cag cca tcc ctc tcc cag gca ctt gtc gca 625
Thr Lys Leu Ser Ile Ser Gln Pro Ser Leu Ser Gln Ala Leu Val Ala

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      gtc att gtc acc cca gcg ggc gag aag ttg ctg cca ttc gcc aaa tcc   721
      Val Ile Val Thr Pro Ala Gly Glu Lys Leu Leu Pro Phe Ala Lys Ser
               65                  70                  75
10
      acc ctt gac gcg gcg gag tct ttc ctc tcc cac gcc aag ggc gcc aac   769
      Thr Leu Asp Ala Ala Glu Ser Phe Leu Ser His Ala Lys Gly Ala Asn
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15    ggt tcg ctc act gga ccg ttg acc gta ggc atc atc ccc acg gcg gct   817
      Gly Ser Leu Thr Gly Pro Leu Thr Val Gly Ile Ile Pro Thr Ala Ala
         95                 100                 105

      cct tac att ttg ccg tca atg ctg tcc atc gtg gat gaa gaa tat cca   865
20    Pro Tyr Ile Leu Pro Ser Met Leu Ser Ile Val Asp Glu Glu Tyr Pro
      110                 115                 120                 125

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      Asp Leu Glu Pro His Ile Val Glu Asp Gln Thr Lys His Leu Leu Ala
25                130                 135                 140

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      Leu Leu Arg Asp Gly Ala Ile Asp Val Ala Met Met Ala Leu Pro Ser
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      gag gca cca ggc atg aag gaa atc ccc ctc tac gac gaa gac ttt atc   1009
      Glu Ala Pro Gly Met Lys Glu Ile Pro Leu Tyr Asp Glu Asp Phe Ile
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      Val Val Thr Ala Ser Asp His Pro Phe Ala Gly Arg Gln Asp Leu Glu
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      cta tcc gcc tta gaa gac ctc gat ctg ctg ctt ctc gac gac gga cac   1105
40    Leu Ser Ala Leu Glu Asp Leu Asp Leu Leu Leu Leu Asp Asp Gly His
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      Cys Leu His Asp Gln Ile Val Asp Leu Cys Arg Arg Gly Asp Ile Asn
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      ccc att agc tcc act act gct gtc acc cgc gca tcc agc ctt acc acc   1201
      Pro Ile Ser Ser Thr Thr Ala Val Thr Arg Ala Ser Ser Leu Thr Thr
            225                 230                 235
50
      gtc atg cag ctc gtc gtc gcc ggc ctt gga tcc acc ttg gtc cca atc   1249
      Val Met Gln Leu Val Val Ala Gly Leu Gly Ser Thr Leu Val Pro Ile
         240                 245                 250

55    agc gca atc cca tgg gaa tgc acc cga cca gga ctg gca aca gcc aac   1297
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      ttc aac tct gat gtc acc gca aac cgc cgc att gga ttg gtg tac cgt   1345
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            305                 310                 315

acc ttg aag caa aat gtc gcg gta gcg cag   taagtttttc tagaggtttt   1491
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        320                 325

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gatc                                                              1675


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Ser Ile Ser Gln Pro Ser Leu Ser Gln Ala Leu Val Ala Leu Glu Thr
        35                  40                  45

Gly Leu Gly Val Gln Leu Ile Glu Arg Ser Thr Arg Lys Val Ile Val
    50                  55                  60

Thr Pro Ala Gly Glu Lys Leu Leu Pro Phe Ala Lys Ser Thr Leu Asp
65                  70                  75                  80

Ala Ala Glu Ser Phe Leu Ser His Ala Lys Gly Ala Asn Gly Ser Leu
                85                  90                  95

Thr Gly Pro Leu Thr Val Gly Ile Ile Pro Thr Ala Ala Pro Tyr Ile
            100                 105                 110

Leu Pro Ser Met Leu Ser Ile Val Asp Glu Glu Tyr Pro Asp Leu Glu
        115                 120                 125

Pro His Ile Val Glu Asp Gln Thr Lys His Leu Leu Ala Leu Leu Arg
    130                 135                 140

Asp Gly Ala Ile Asp Val Ala Met Met Ala Leu Pro Ser Glu Ala Pro
145                 150                 155                 160
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Gly Met Lys Glu Ile Pro Leu Tyr Asp Glu Asp Phe Ile Val Val Thr
                165                 170                 175

Ala Ser Asp His Pro Phe Ala Gly Arg Gln Asp Leu Glu Leu Ser Ala
            180                 185                 190

Leu Glu Asp Leu Asp Leu Leu Leu Leu Asp Asp Gly His Cys Leu His
        195                 200                 205

Asp Gln Ile Val Asp Leu Cys Arg Arg Gly Asp Ile Asn Pro Ile Ser
    210                 215                 220

Ser Thr Thr Ala Val Thr Arg Ala Ser Ser Leu Thr Thr Val Met Gln
225                 230                 235                 240

Leu Val Val Ala Gly Leu Gly Ser Thr Leu Val Pro Ile Ser Ala Ile
                245                 250                 255

Pro Trp Glu Cys Thr Arg Pro Gly Leu Ala Thr Ala Asn Phe Asn Ser
            260                 265                 270

Asp Val Thr Ala Asn Arg Arg Ile Gly Leu Val Tyr Arg Ser Ser Ser
        275                 280                 285

Ser Arg Ala Glu Glu Phe Glu Gln Phe Ala Leu Ile Leu Gln Arg Ala
    290                 295                 300

Phe Gln Glu Ala Val Ala Leu Ala Ala Ser Thr Gly Ile Thr Leu Lys
305                 310                 315                 320

Gln Asn Val Ala Val Ala Gln
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```

Patent Claims

1. An isolated polynucleotide from coryneform bacteria, comprising a polynucleotide sequence chosen from the group consisting of

a) polynucleotide which is identical to the extent of at least 70 % to a polynucleotide which codes for a polypeptide which comprises the amino acid sequence of SEQ ID No. 2,

b) polynucleotide which codes for a polypeptide which comprises an amino acid sequence which is identical to the extent of at least 70 % to the amino acid sequence of SEQ ID No. 2,

c) polynucleotide which is complementary to the polynucleotides of a) or b), and

d) polynucleotide comprising at least 15 successive nucleotides of the polynucleotide sequence of a), b) or c),

the polypeptide preferably having the activity of the transcription regulator OxyR.

2. A polynucleotide as claimed in claim 1, wherein the polynucleotide is a preferably recombinant DNA which is capable of replication in coryneform bacteria.

3. A polynucleotide as claimed in claim 1, wherein the polynucleotide is an RNA.

4. A polynucleotide as claimed in claim 2, comprising the nucleic acid sequence as shown in SEQ ID No. 1.

5. DNA as claimed in claim 2 which is capable of replication, comprising

(i) the nucleotide sequence shown in SEQ ID No. 1, or

(ii) at least one sequence which corresponds to sequence (i) within the range of the degeneration of the genetic code, or

(iii) at least one sequence which hybridizes with the sequence complementary to sequence (i) or (ii), and optionally

(iv) sense mutations of neutral function in (i).

6. A polynucleotide sequence as claimed in claim 2, which codes for a polypeptide which comprises the amino acid sequence shown in SEQ ID No. 2.

7. Coryneform bacteria in which the oxyR gene is enhanced, in particular over-expressed.

8. A process for the fermentative preparation of L-amino acids, in particular L-lysine, which comprises carrying out the following steps:

a) fermentation of the coryneform bacteria which produce the desired L-amino acid and in which at least the oxyR gene or nucleotide sequences which code for it are enhanced, in particular over-expressed;

b) concentration of the L-amino acid in the medium or in the cells of the bacteria, and

c) isolation of the L-amino acid.

9. A process as claimed in claim 8,
wherein
bacteria in which further genes of the biosynthesis pathway of the desired L-amino acid are additionally enhanced are employed.

10. A process as claimed in claim 8,
wherein

bacteria in which the metabolic pathways which reduce the formation of the desired L-amino acid are at least partly eliminated are employed.

11. A process as claimed in claim 8, wherein a strain transformed with a plasmid vector is employed, and the plasmid vector carries the nucleotide sequence which codes for the oxyR gene.

12. A process as claimed in claim 8, wherein the expression of the polynucleotide which codes for the oxyR gene is enhanced, in particular over-expressed.

13. A process as claimed in claim 8, wherein the regulatory properties of the polypeptide (enzyme protein) for which the polynucleotide oxyR codes are increased.

14. A process as claimed in claim 8, wherein for the preparation of L-amino acids, in particular L-lysine, coryneform microorganisms in which at the same time one or more of the genes chosen from the group consisting of

14.1 the dapA gene which codes for dihydrodipicolinate synthase,

14.2 the gap gene which codes for glycerolaldehyde 3-phosphate dehydrogenase,

14.3 the tpi gene which codes for triose phosphate isomerase,

14.4 the pgk gene which codes for 3-phosphoglycerate kinase,

14.5 the pyc gene which codes for pyruvate carboxylase,

14.6 the lysE gene which codes for lysine export,

14.7 the mqo gene which codes for malate-quinone oxidoreductase,

14.8 the zwf gene which codes for glucose 6-phosphate dehydrogenase,

14.9 the gnd gene which codes for 6-phosphogluconate dehydrogenase,

14.10 the sod gene which codes for superoxide dismutase,

14.11 the zwa1 gene which codes for the Zwa1 protein,

14.12 the lysC gene which codes for a feed back resistant aspartate kinase,

is or are enhanced or over-expressed are fermented.

15. A process as claimed in claim 8, wherein for the preparation of L-amino acids, in particular L-lysine, coryneform microorganisms in which at the same time one or more of the genes chosen from the group consisting of

15.1 the pck gene which codes for phosphoenol pyruvate carboxykinase,

15.2 the pgi gene which codes for glucose 6-phosphate isomerase

15.3 the poxB gene which codes for pyruvate oxidase,

15.4 the zwa2 gene which codes for the Zwa2 protein

is or are attenuated are fermented.

16. Coryneform bacteria which contain a vector which carries a polynucleotide as claimed in claim 1.

17. A process as claimed in one or more of the preceding claims,
wherein
microorganisms of the genus Corynebacterium are employed.

18. A process for discovering RNA, cDNA and DNA in order to isolate nucleic acids, or polynucleotides or genes which code for the transcription regulator OxyR or have a high similarity with the sequence of the oxyR gene, which comprises employing the polynucleotide sequences as claimed in claims 1, 2, 3 or 4 are employed as hybridization probes.

19. A process as claimed in claim 18,
wherein
the hybridization is carried out under a stringency corresponding to at most 2x SSC.

Abstract

The invention relates to an isolated polynucleotide comprising a polynucleotide sequence chosen from the group consisting of

a) polynucleotide which is identical to the extent of at least 70 % to a polynucleotide which codes for a polypeptide which comprises the amino acid sequence of SEQ ID No. 2,

b) polynucleotide which codes for a polypeptide which comprises an amino acid sequence which is identical to the extent of at least 70 % to the amino acid sequence of SEQ ID No. 2,

c) polynucleotide which is complementary to the polynucleotides of a) or b), and

d) polynucleotide comprising at least 15 successive nucleotides of the polynucleotide sequence of a), b) or c),

and a process for the fermentative preparation of L-amino acids using coryneform bacteria in which at least the oxyR gene is present in enhanced form, and the use of the polynucleotide sequences as hybridization probes.

Figure 1: Map of the plasmid pEC-T18mob2

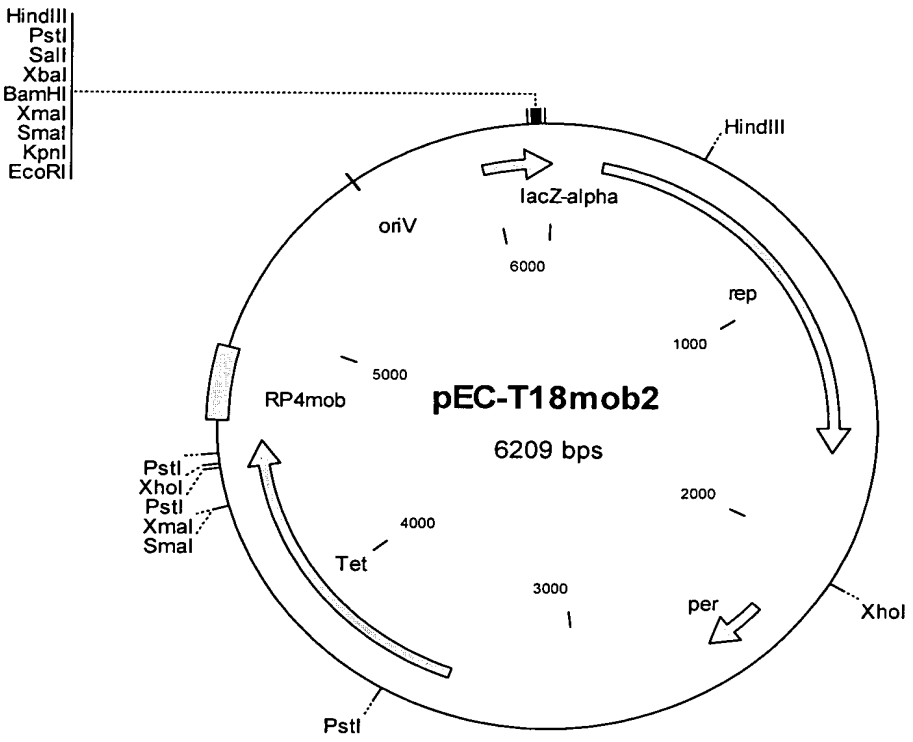

Figure 2: Map of the plasmid pT-oxyRexp